Impact of Drug-Like Properties on Hit Selection and Optimization for Quality Clinical Candidates

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Chemical and Screening Sciences
Wyeth Research

NIH-NIAID Conference
Optimizing Positive Hits for Potency and Safety
February 7, 2007

Preferred Drug Characteristics

- **Oral administration**
  - Use outside hospital
  - Wide patient population
  - Extended treatment period
  - Requires intestinal absorption
- **Once per day dose**
  - Patient compliance
  - Patient comfort
  - Requires sufficient half-life
- **If not achievable: alternate dosing approach**

Growing Attention to Properties in Discovery

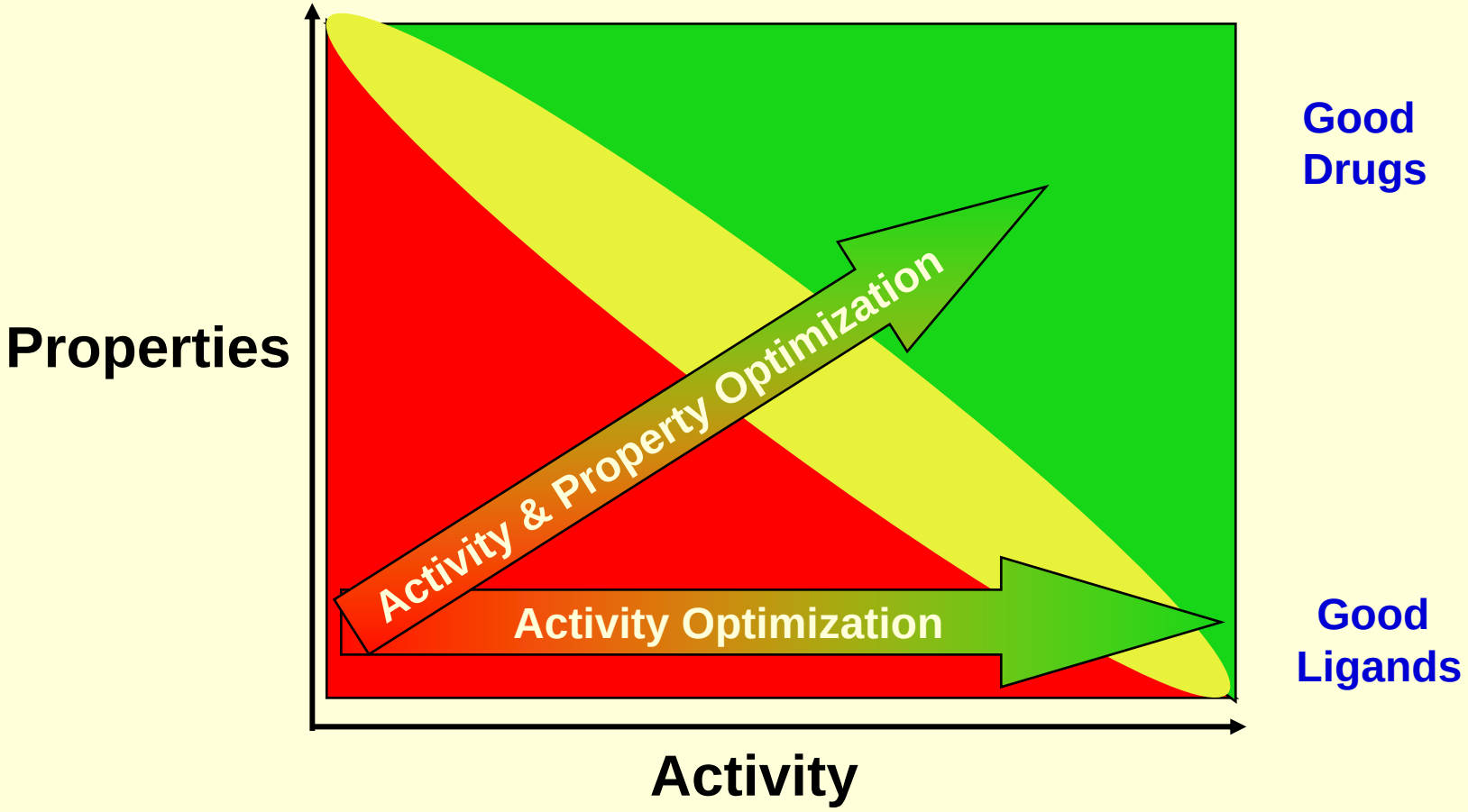
- **Development failure due to properties**
  - Prentis RA; *et al.* (1988) *Br J Clin Pharmacol* 25:387-396
  - Kennedy, T (1997) *Drug Discovery Today* 2:436-444
    - **39% failed due to poor biopharmaceutical properties**
    - 21% failed due to animal toxicity or human adverse effects
- ***In vitro* property assays developed; Example: Caco-2**
  - Hidalgo, IJ; Raub, T., Borchardt, R (1989) *J Gastroenterology 96:609-616*
- **Physicochemical guides for medicinal chemists**
  - Lipiniski, CA; *et al.* (1997) *Adv. Drug Delivery Rev. 23*, 3-25
  - Rule of 5 revolution
- **Property-based structure design**
  - van de Waterbeemd, H; *et al.* (2001) *J. Med. Chem*. *44*, 1313-1332
  - Structure modification to optimize properties

Importance of Properties in Discovery

"Although small molecules that exhibit potent antimalarial properties are regularly discovered in various screening programs, most of these compounds will never reach clinical use, primarily because of poor pharmacokinetic and/or toxicity profiles."

- ***The Role of In Vitro ADME Assays in Antimalarial Drug Discovery and Development***
  - Todd Schearer, Kirsten Smith, Damaris Diaz, Constance Asher, Julio Ramirez
  - Walter Reed Army Institute of Research
  - *Comb. Chem. HTS* (2005), 8:89-98

Changing Criteria for Clinical Candidates



Balance activity and drug-like properties

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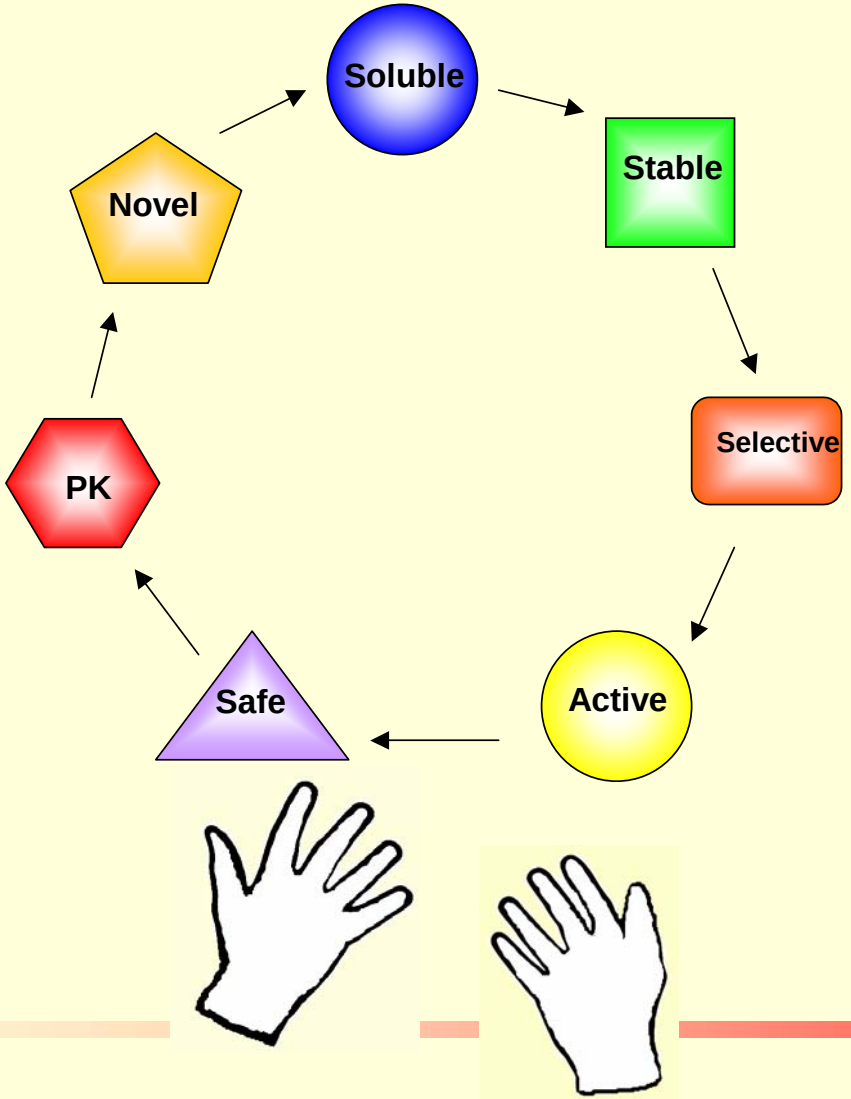
Drug Discovery is a Juggling Act
Dynamic Process of Co-Optimization



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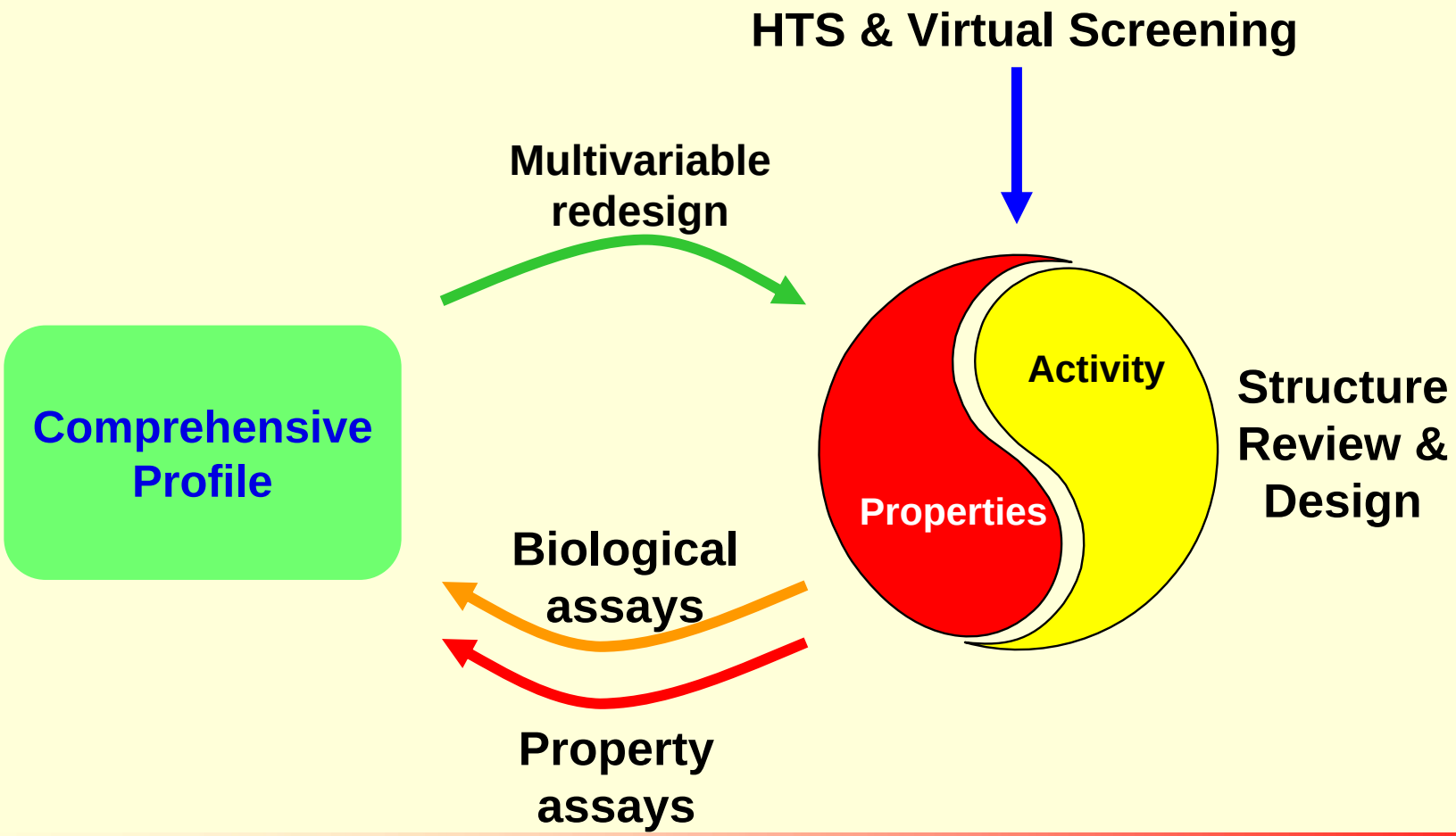
Resources for Property Improvement

- ***In silico*** **tools**
- **Rules (e.g., Lipinski)**
- ***In vitro*** **pharmaceutical property profiling**
- **Discovery pharmaceutics and formulation**
- ***In vivo*** **pharmacokinetics**
- **Discovery toxicology**

Current Discovery Property Strategy

- **Measure** drug-like properties for all compounds
- **Select** leads from HTS “hits” using properties
- **Optimize** leads using properties
- **Diagnose** PK limitations using properties
- **Advance** clinical candidates using property criteria
- **Apply** properties to biological assays

Property-Based Selection and Optimization



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Impact of Drug-like Property Attention

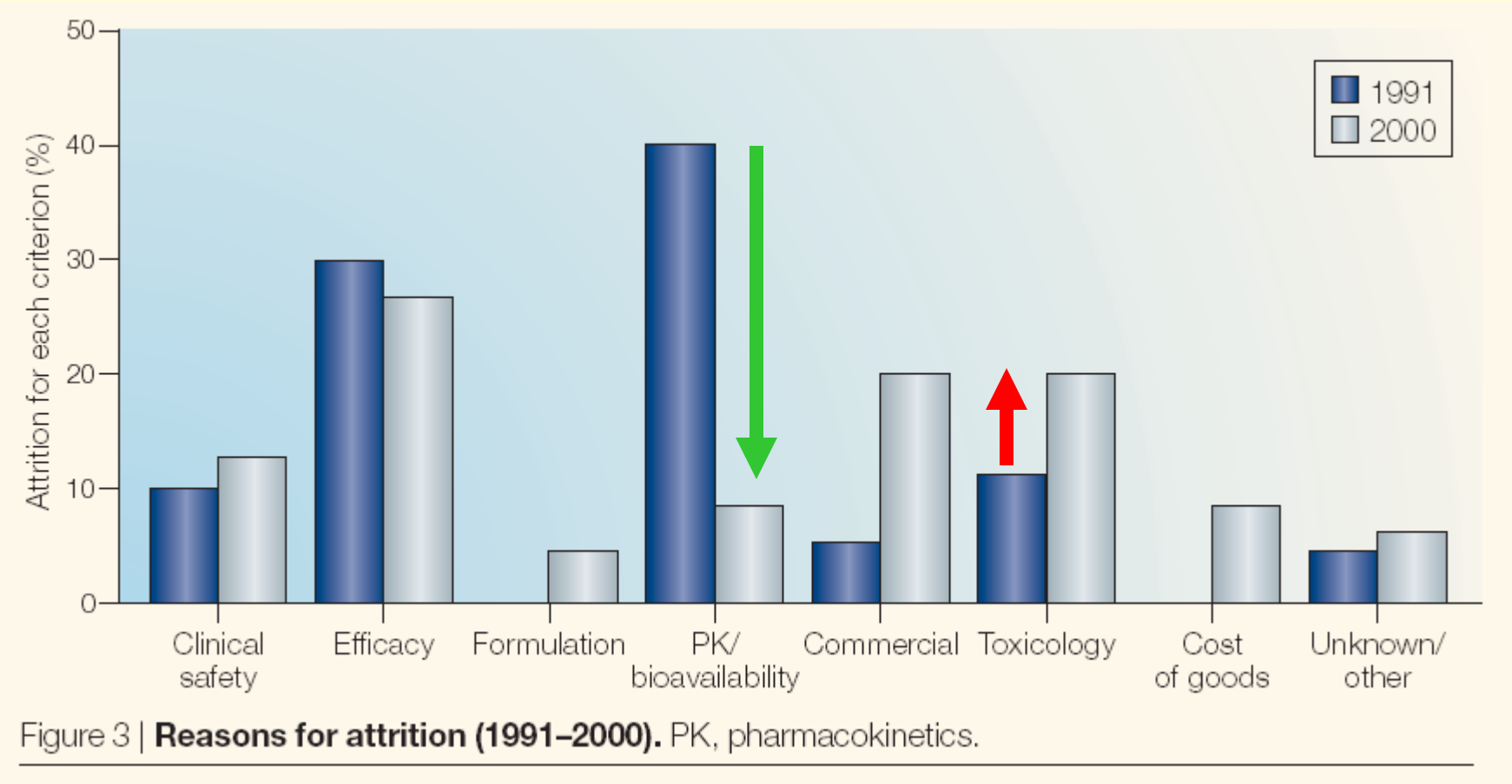

Figure 3 | **Reasons for attrition (1991–2000).** PK, pharmacokinetics.

Kola I, Landis J (2004) "Can the Pharmaceutical Industry Reduce Attrition Rates"
Nature Reviews Drug Discovery 3: 711-715

PK attrition down, Safety/Tox attrition continue

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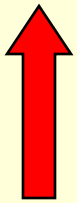
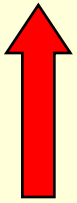
Drug-Like Properties

Pharmacological Properties:
Pharmacokinetics, Pharmacodynamics, Toxicology



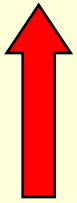
Living System

Physicochemical Properties:
Solubility, Permeability, Chemical Stability



Physical Environment

Biochemical Properties:
Metabolism, Transporter Affinity, Target Affinity



Proteins

Structural / Molecular Properties:
MW, H-bonds, Lipophilicity, PSA, pKa, Shape, Reactivity

Properties result from structure

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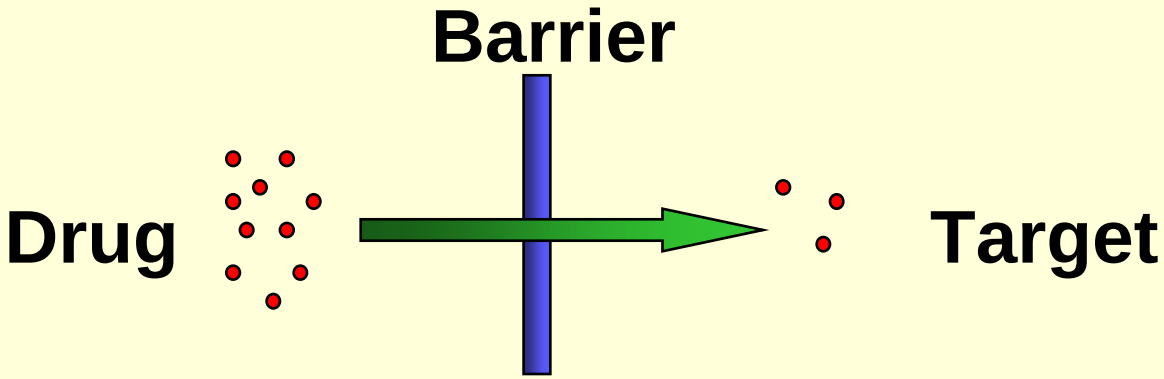
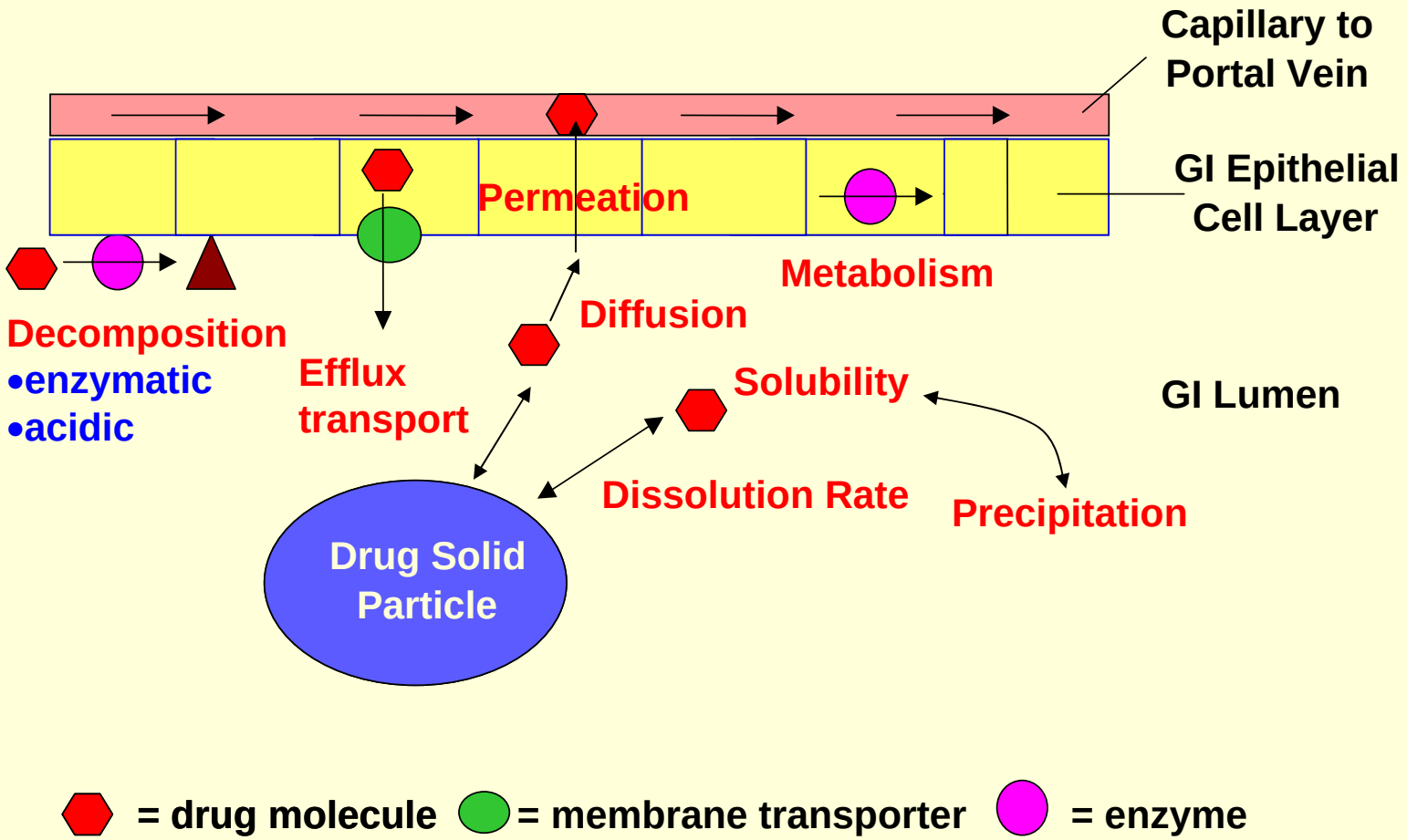
Barriers *In Vivo*



Example: Barriers in Gastrointestinal Tract
Opposition to Absorption



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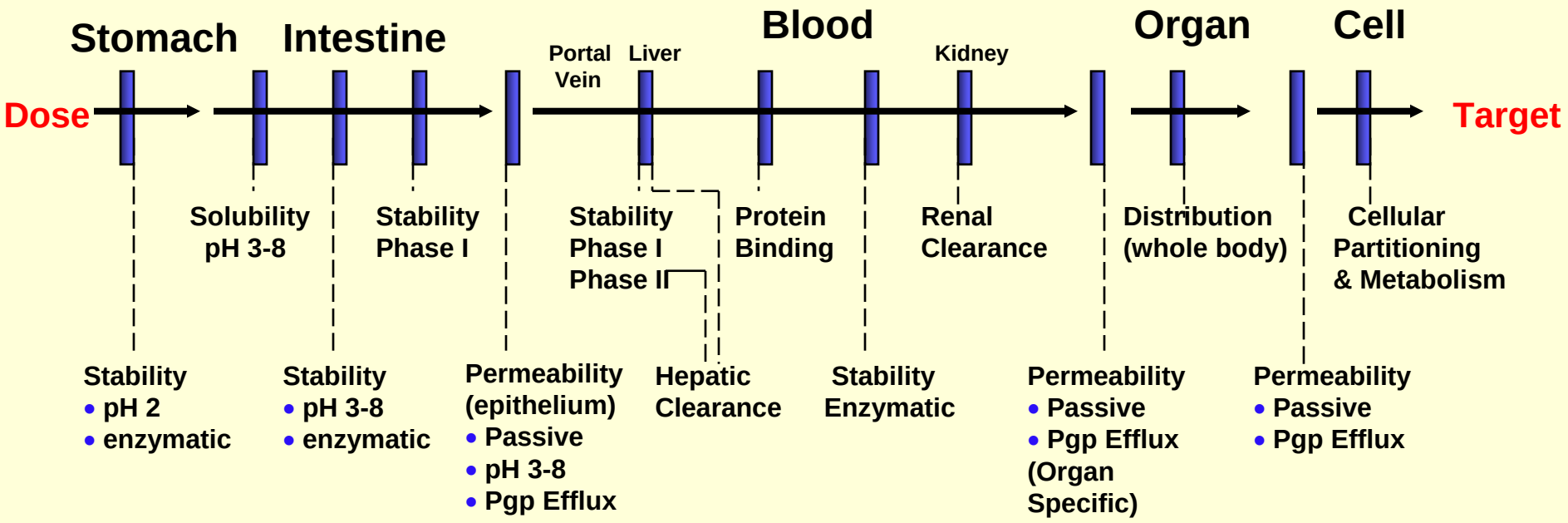
Drugs Must Survive *In Vivo* Barriers



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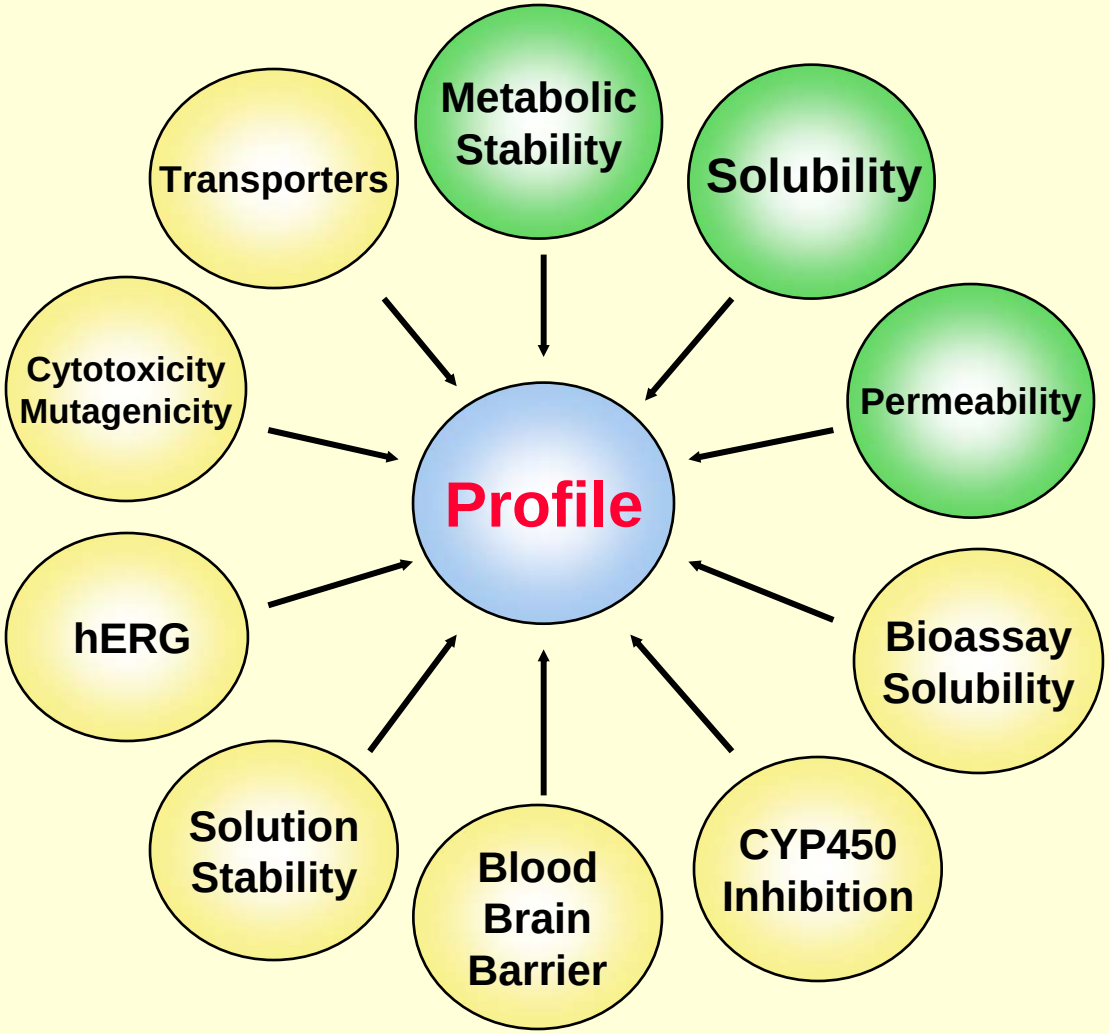
Property Profiling Assays
Examples



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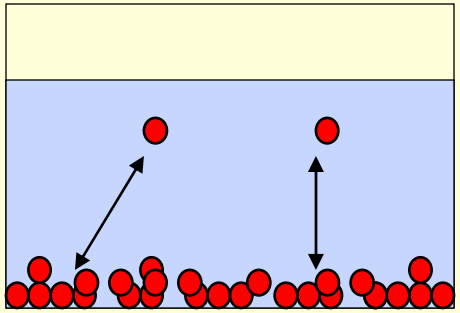
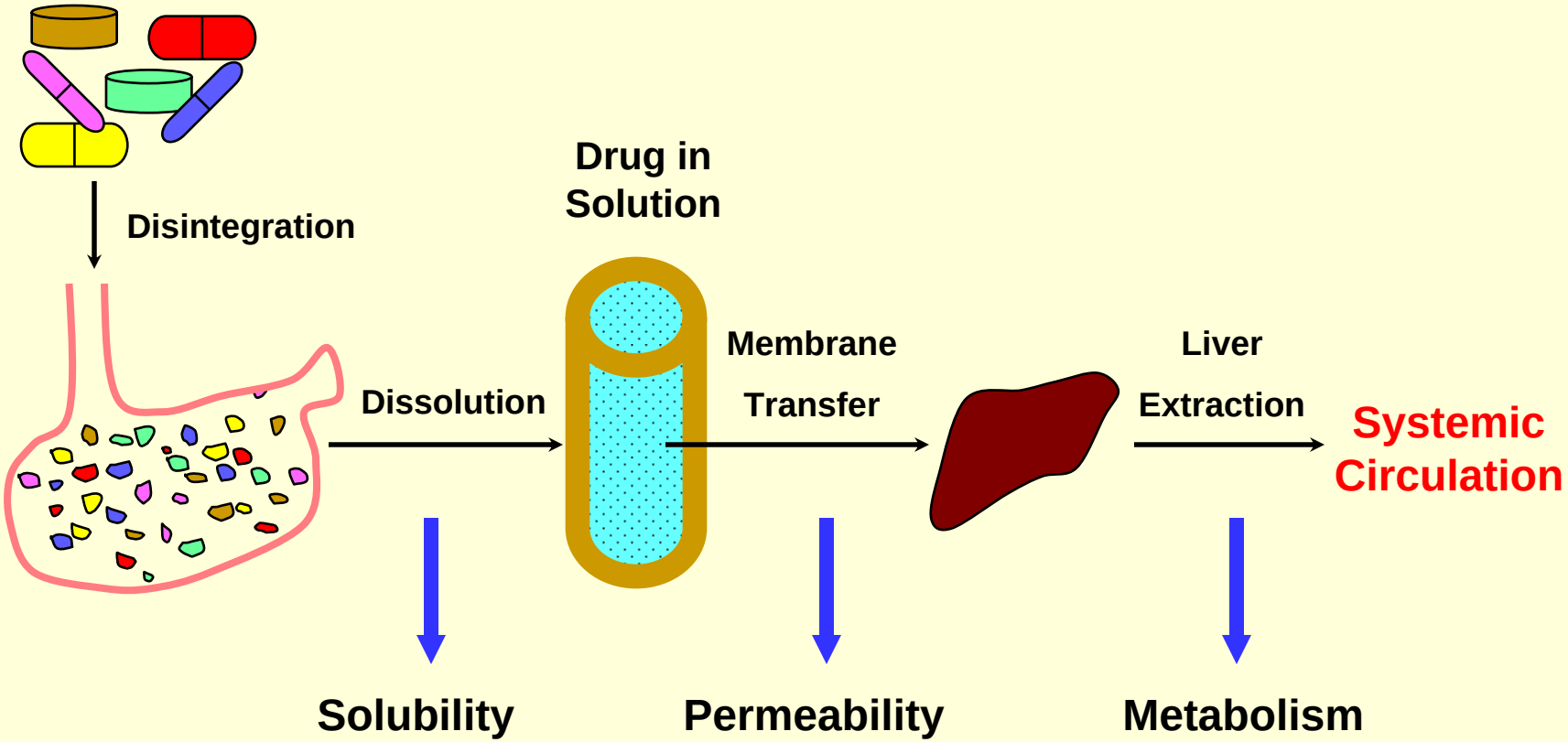
SOLUBILITY



Solubility, Permeability and Metabolic Stability Affect Oral Bioavailability



Solubility affects oral absorption and bioavailability

Courtesy of Li Di

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Solubility Issues *In Vivo*

- **Low solubility limits GI absorption**
- **Poor oral bioavailability**
- **Abnormal PK profile**
- **Inter-subject, -species variation**
- **Problematic formulation**
  - Toxicity of vehicles, prodrug approach
- **Expensive and prolonged development**
- **Burden to patients**
  - Amprenavir: 8 capsules b.i.d

Courtesy of Li Di

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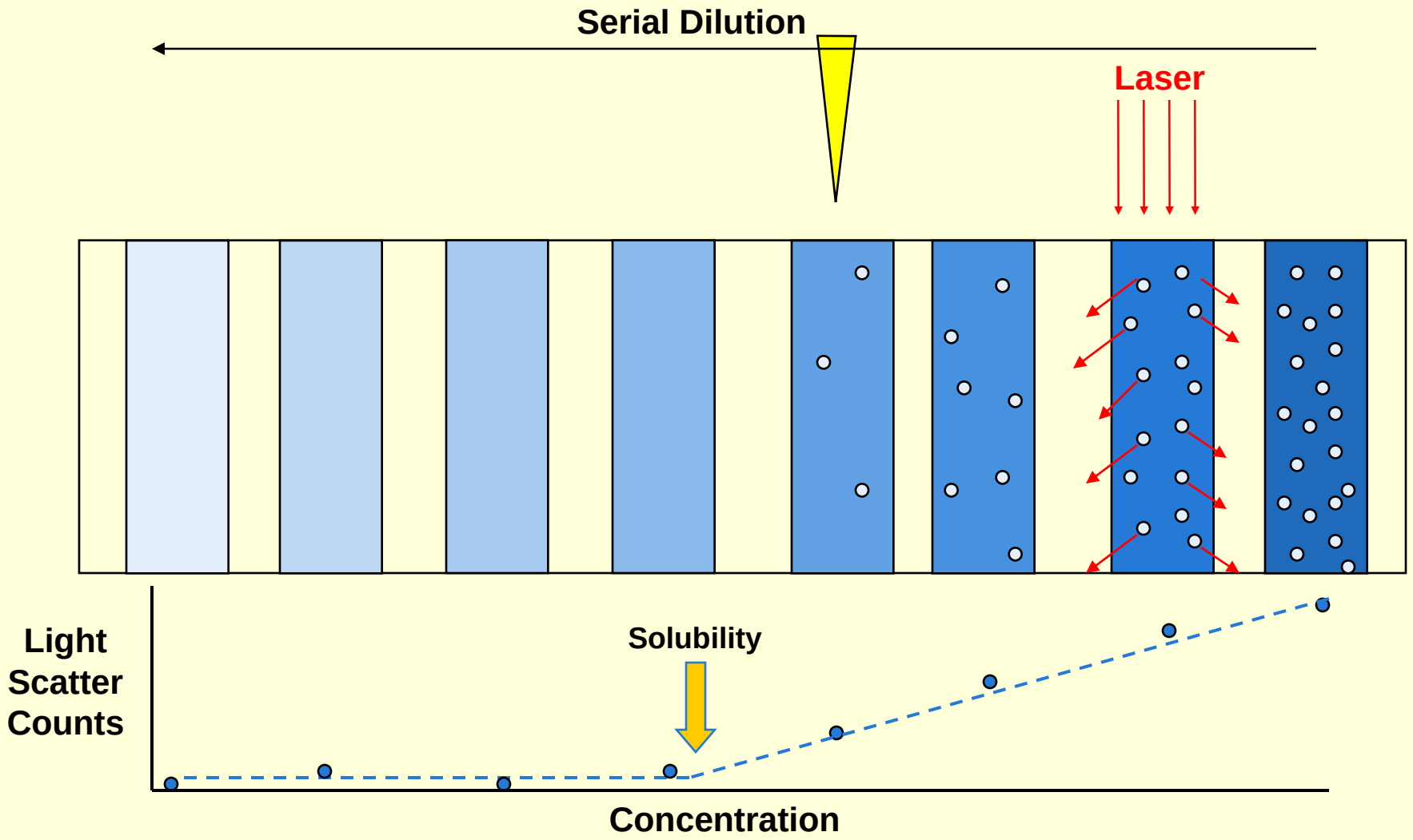
Kinetic vs. Thermodynamic Solubility

- **Kinetic Solubility: dissolve first in DMSO, add to buffer**
- **Thermodynamic Solubility: add buffer to solid**

- **Kinetic solubility is relevant to drug discovery**
  - All experiments are from DMSO stock solutions

- **Thermodynamic solubility is not relevant for discovery**
  - Solubility of amorphous or variable crystalline solids highly variable
  - Thermodynamic solubility is relevant to dosage form in Development
  - Solid form will change during Development

Measurement of Kinetic Solubility
Nephelometric Method



Laser System: BMG Lab Technologies Flow Cytometry System: BD Gentest

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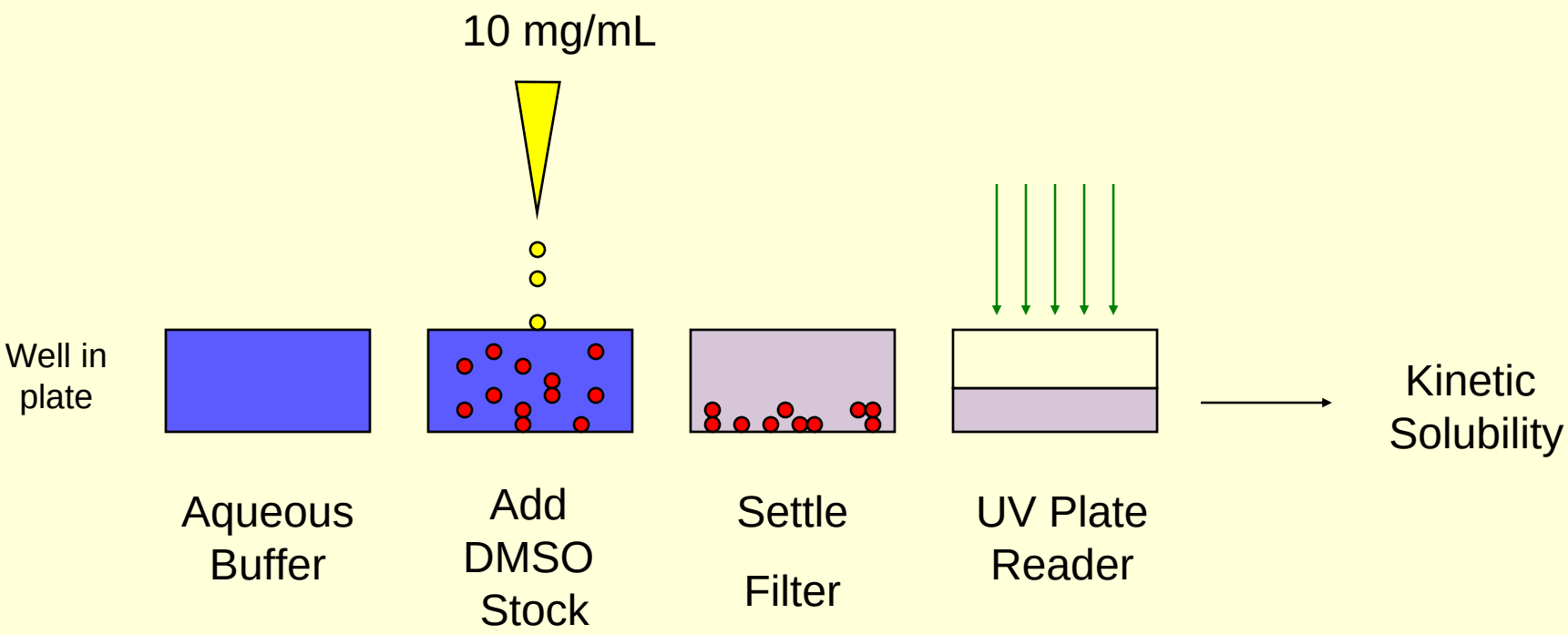
Measurement of Kinetic Solubility
Direct UV Method



96-well format

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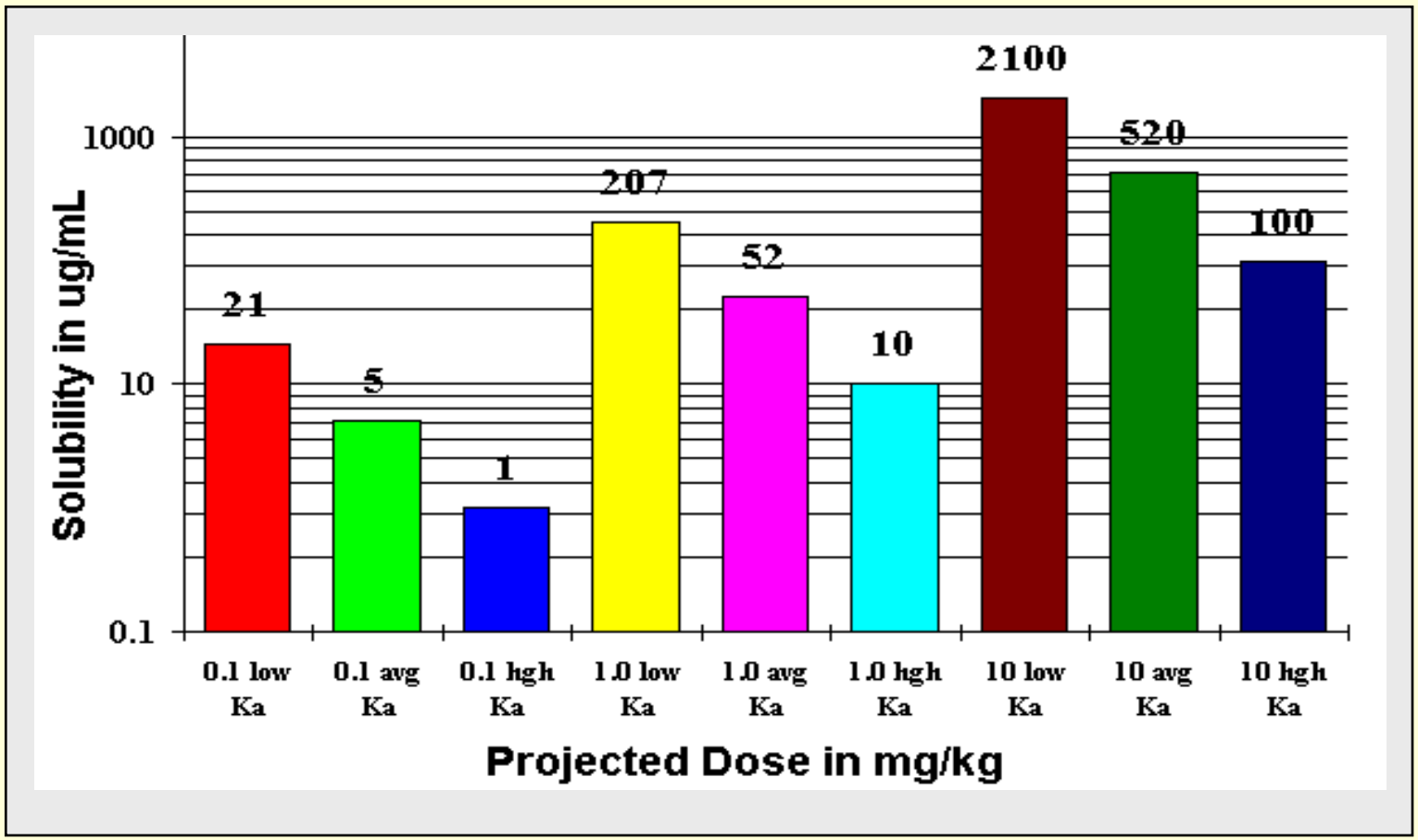
Minimum Acceptable Solubility (μg/mL)

Minimum solubility for low, medium and high permeability (Ka) compounds.
Example: at 1 mg/Kg dose and moderate permeability, you need 52 ug/mL solubility.



C. Lipinski, et al, Adv. Drug Delivery Review, 1997, 23, 3-35

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Solubility Classification for Screening

Classification	Solubility
High	60 ug/mL
Moderate	10-60 ug/mL
Low	10 ug/mL

- Classification for screening
- "High" might not be sufficient for animal dosing
- "High" is different than in BCS Classification

C. Lipinski, et al, Adv. Drug Delivery Review, 1997, 23, 3-35

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Target Solubility for Animal Dosing

Dose (mpk)	Target Solubility (mg/mL)	
	P.O.	I.V.
1	0.1-0.2	0.2-1
5	0.5-1	1-5
10	1-2	2-10
Ideal Volume (mL/Kg)	5-10	1-5

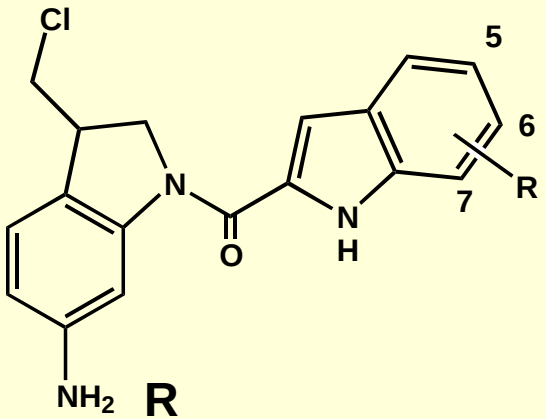
Data calculated based on 250 g rat

Courtesy of Li Di

Structure Modification for Solubility

- **Solubility**
  - Chemical modification
    - Add ionizable group
    - Add polar group
    - Remove unnecessary lipophilic group
  - Reduce crystal packing energy
    - Out of plane substitutions
  - Prodrug
- **Dissolution Rate**
  - Reduce particle size – increase surface area
  - Oral solution
  - Surfactants – improve wetting
  - Salt form

Example: Improve Solubility and Retain Activity



Improve solubility and cytotoxicity (oncology)

R	Solubility (μM)	IC50 (μM) AA8	UV4	EMT6	SKOV3
5,6,7-triOMe	32	0.35	0.055	0.27	0.63
5-OMe	23	0.31	0.047	0.23	0.67
5-O$(CH_2)_2NMe_2$	700	0.16	0.044	0.12	0.26
5-OMe, 6-O$(CH_2)_2NMe_2$	>1200	0.22	0.039	0.11	0.15
5-OMe, 7-O$(CH_2)_2NMe_2$	47	0.14	0.029	0.09	0.16

J. B. J. Milbank, et al, J. Med. Chem., 1999, 42, 649-658

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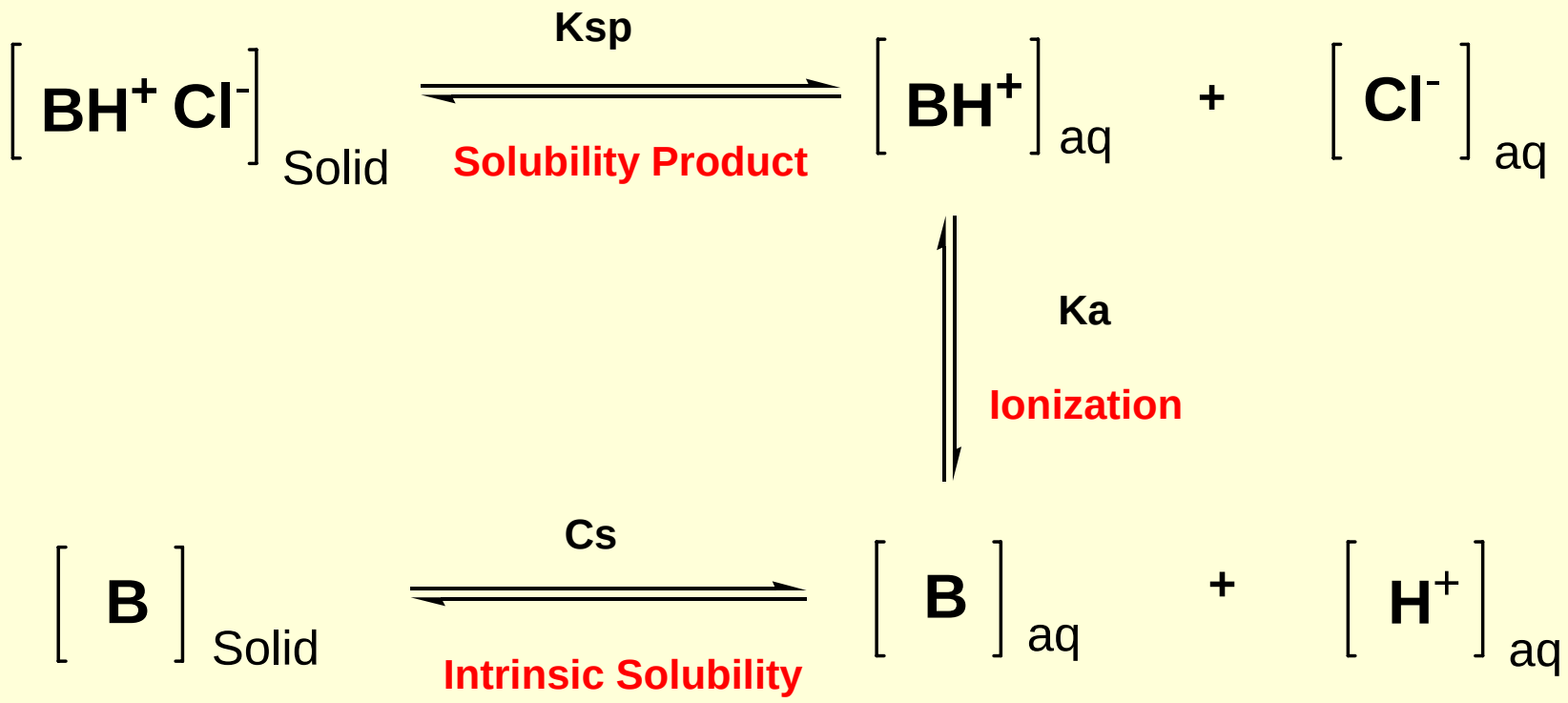
Salt Form Equilibria



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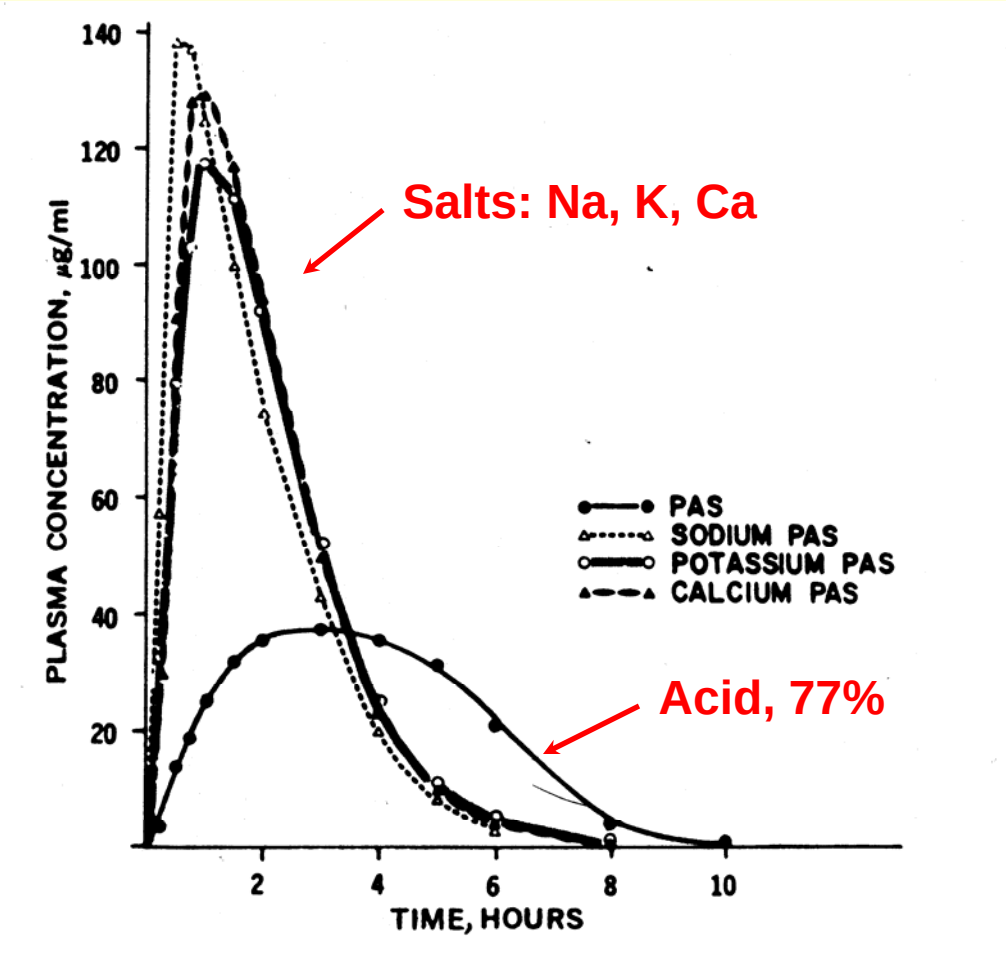
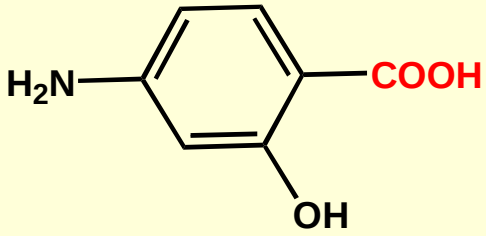
Salt Form to Increase Absorption





Salts

- Increase dissolution
- Slow precipitation
- Precipitates as amorphous

S.H. Wan, et al, J. Pharm. Sci., 1974, 63, 708-711

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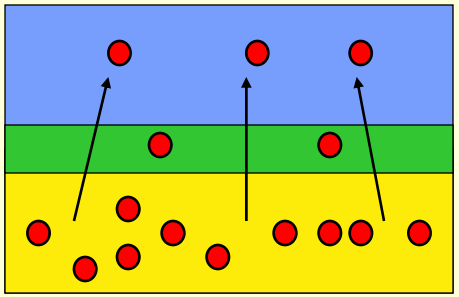
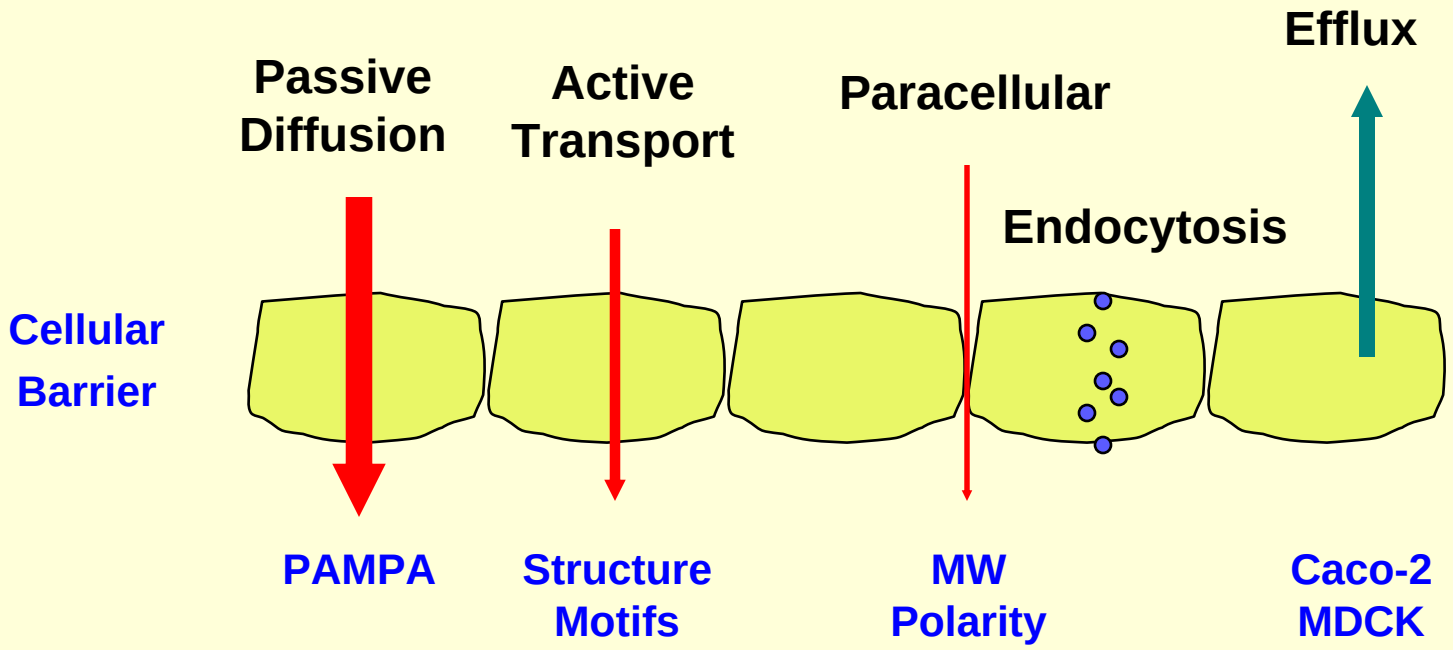
PERMEABILITY



Permeability

- **Compound flux through a lipid membrane**
- **Important for:**
  - Absorption – Intestine (orally delivered drugs)
  - Organ barriers (e.g., BBB)
  - Cells – *In vivo* tissue with target
  - Cells – *In vitro* biological assay
- **95% of commercial drugs are primarily absorbed by passive diffusion**

Permeation Mechanisms



Passive diffusion: major absorption pathway

Li Di, *et al.*, Eur. J. Med. Chem., 2003, 38, 223-232

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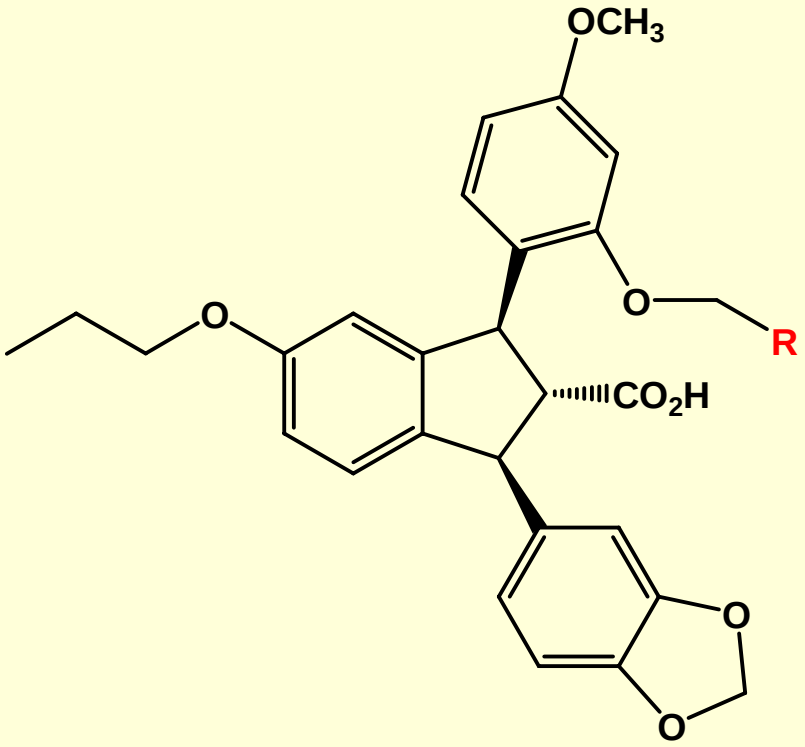
Structural Modifications to Improve Permeability

- Optimize lipophilicity (Log $D_{7.4}$ 1-3)
- Reduce hydrogen bonds
- Reduce polarity
- Reduce molecular weight (if high)
- Reduce rotatable bonds
- Remove carboxylic acid for brain penetration
- Prodrug approach

Li Di, *et al. Current Opin. in Drug Discovery & Dev.* (2005), 8(4), 495-504

Predict Oral Absorption with Caco-2



R	ETA, Ki (nM)	Caco-2 (cm/h)	%F (rat)
CO_2H	0.43	0.0075	4
CH_2OH	1.1	0.2045	66

C. P. Lee, et al, Adv. Drug Delivery Rev., 1997, 23, 99-109

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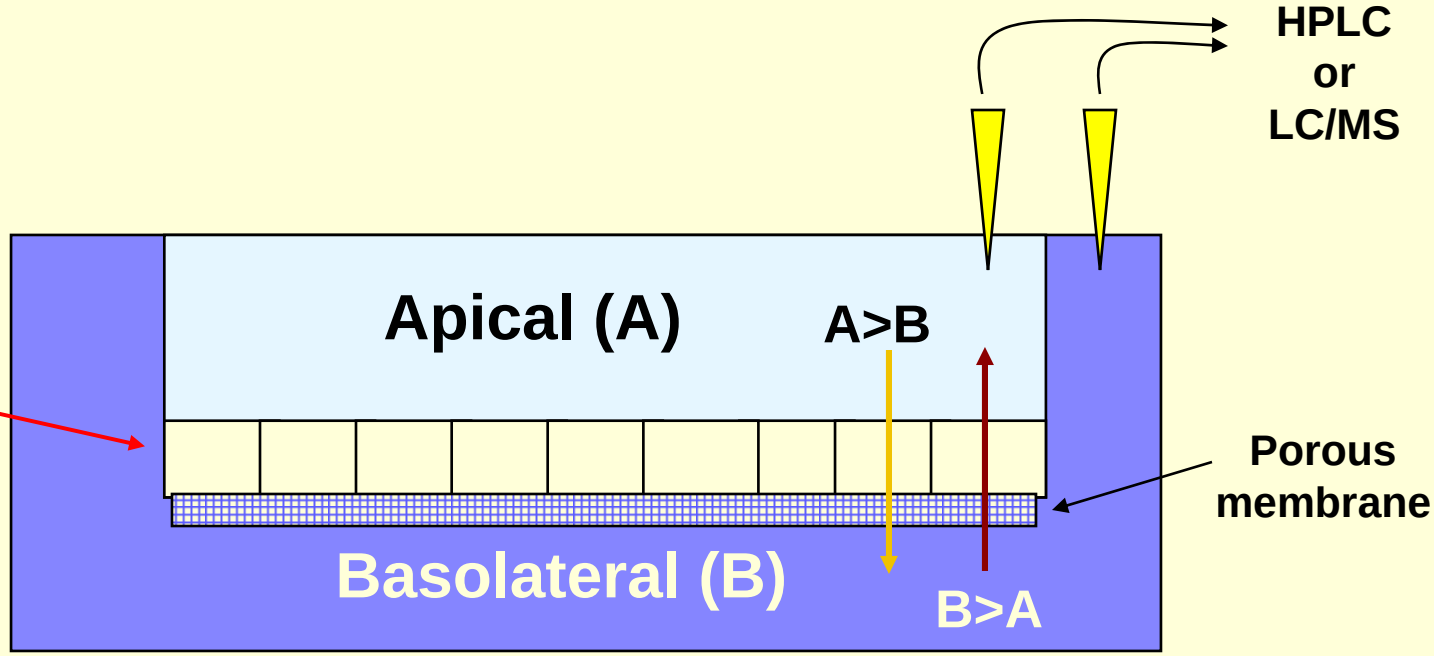
Cell Monolayer Method

Well of 24 or 96
"Transwell" plate



- 1-2 hr incubation

Transporter Information:

- "Efflux Ratio" = B>A / A>B
- Inhibitor to (e.g., Pgp with verapamil)

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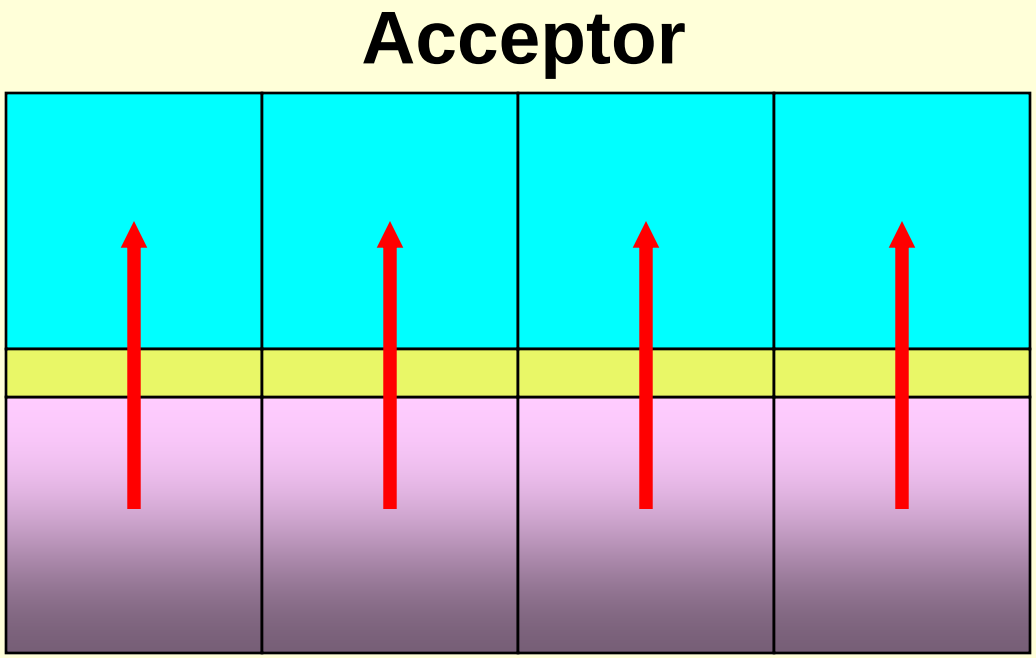
PAMPA Method

"Parallel Artificial Membrane Permeability Assay"



Buffer (pH 7.4)

Lipid on Filter Plate (2% Phosphatidyl Choline in dodecane)

Drug in Buffer (25μg/mL, pH 7.4)

Measures Passive Diffusion

Manfred Kansy, *et al.*, *J Med Chem* (1998) 41, 1007

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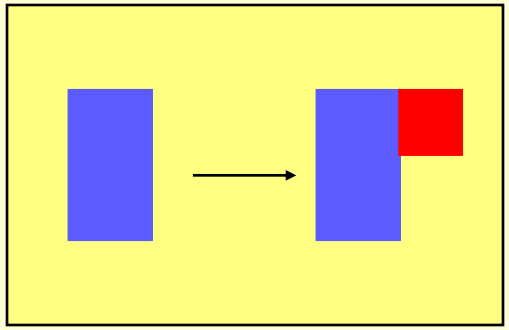
Metabolic Stability



Using Screening Data to Guide Synthetic Modification and Lead to More Stable Compounds

Start, $t_{1/2}$ (min)	
Rat	Mouse
5	10
7	7
5	5
7	8
3	2
8	5
5	3

3 months later, $t_{1/2}$ (min)		
Rat	Mouse	Human
>30	12	>30
>30	29	>30
20	10	18
>30	14	>30
12	30	>30
6	10	>30
>30	13	>30

- **High throughput: 300-500 / week vs. 20 / week**
- **Fast turnaround: 1-2 weeks**
- **Parallel optimization**

Courtesy of Li Di

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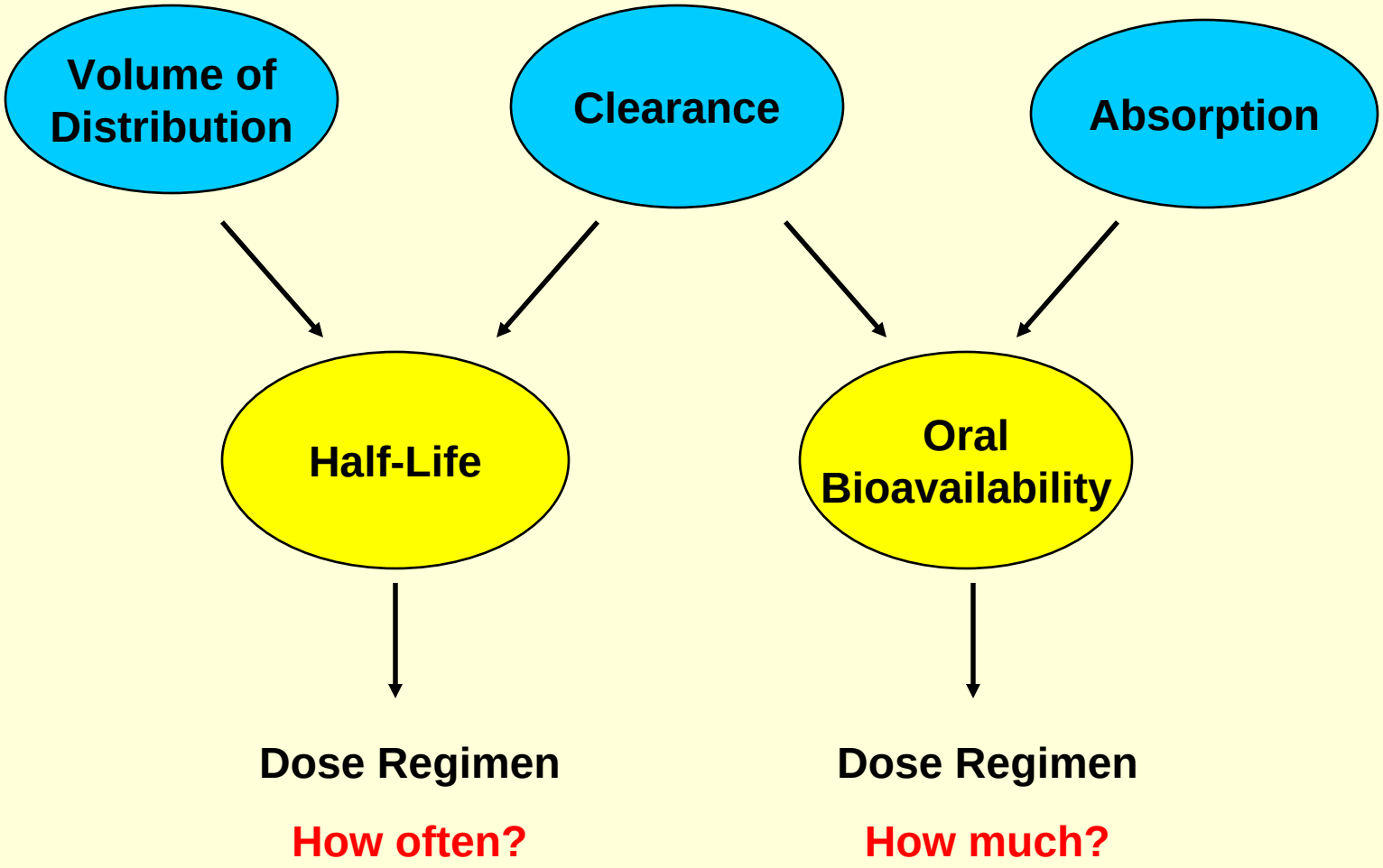
Impact of Metabolism on Pharmacokinetics



H. Van de Waterbeemd, et al, Nat. Rev. Drug Dis., 2003, 2, 192-204

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Correlation between *in Vitro* Metabolic Stability and *in Vivo* PK Data

Compound	In vitro $t_{1/2}$ (min)	In vivo CL (ml/min/kg)	% F Rat
1	5	53	3
2	6	55	8
3	7	49	15
4	14	18	20
5	> 30	14	41

Compounds with short half-life tend to have high clearance and low oral bioavailability

Courtesy of Li Di

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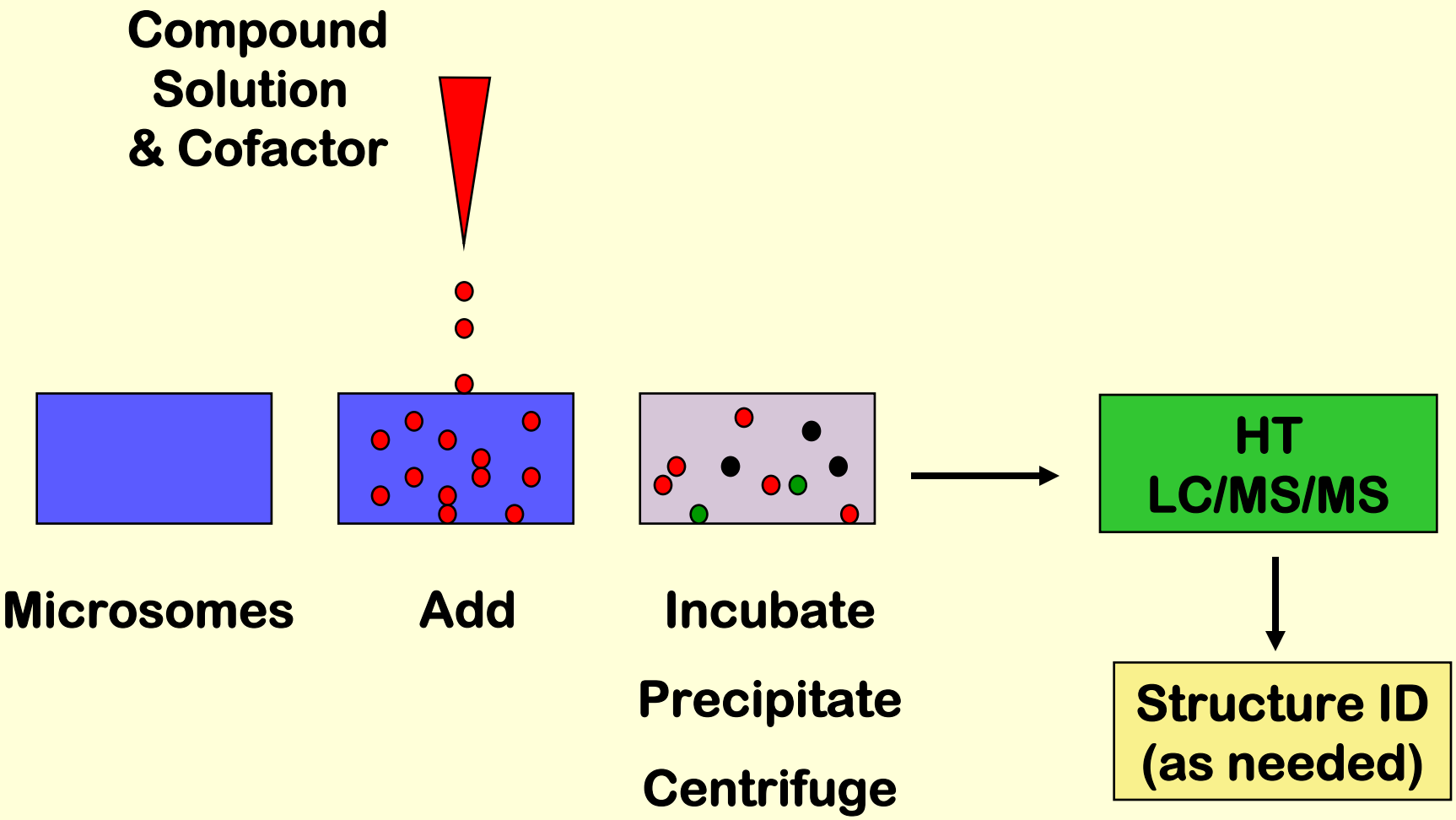
Stability Profile Overview



96 Well Plate Format

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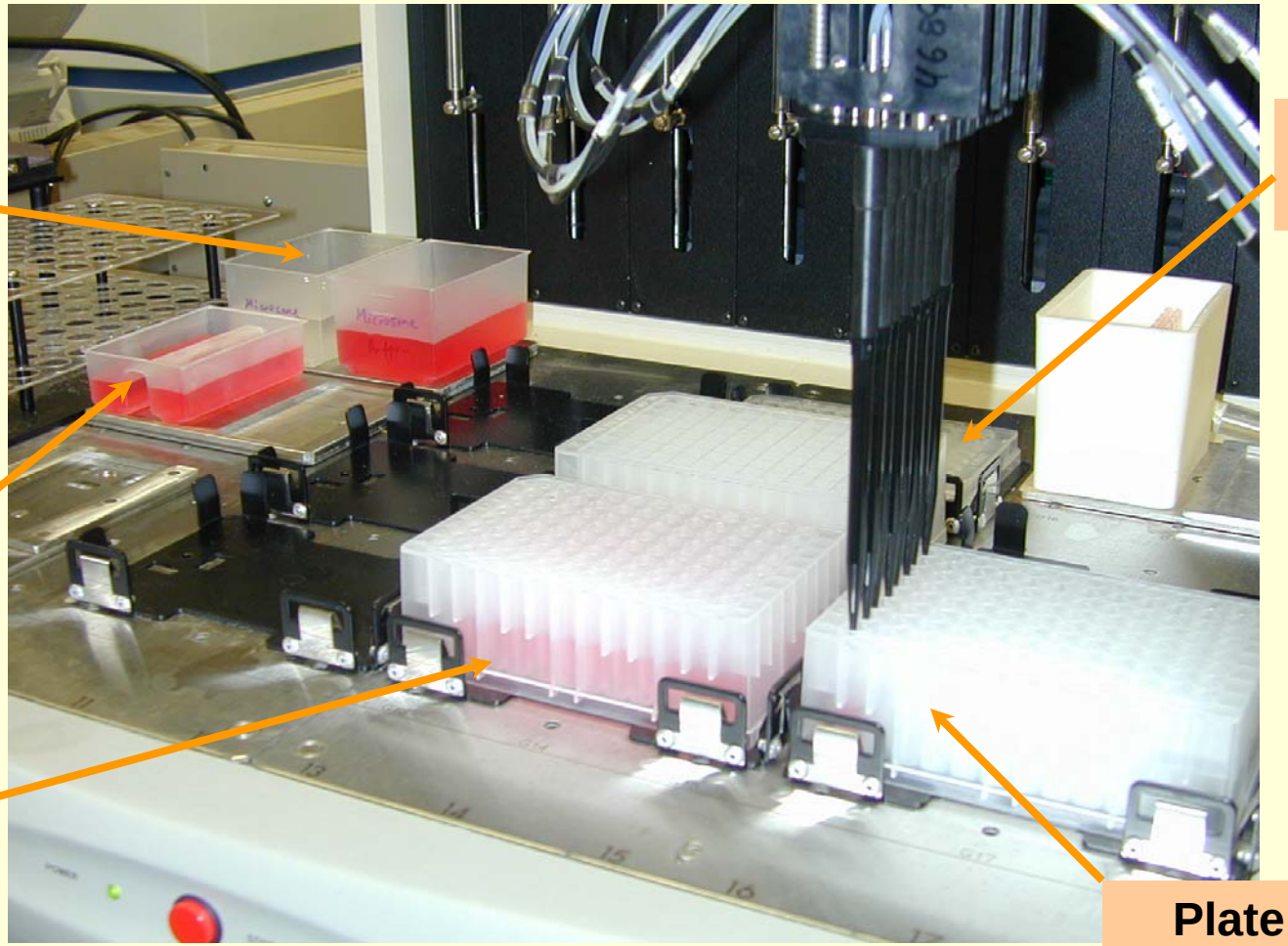
Microsomal Stability Assay with Packard Robot



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High Throughput LC-MS-MS System

Software Control & Reporting

"QuanOptimize" MS/MS Method Development

12 Plate Autosampler

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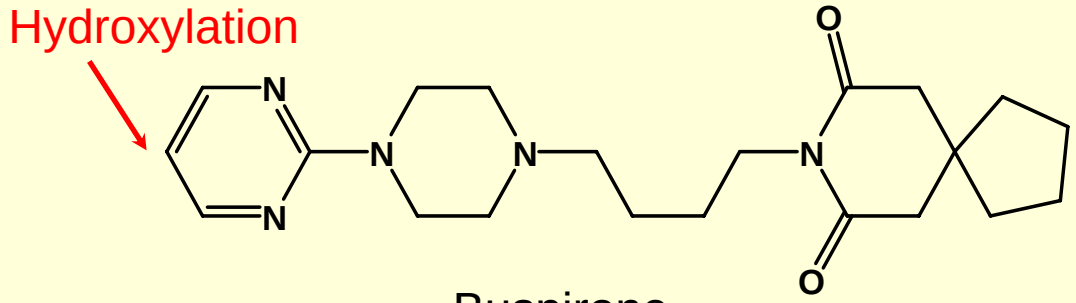
Strategies to Enhance Metabolic Stability

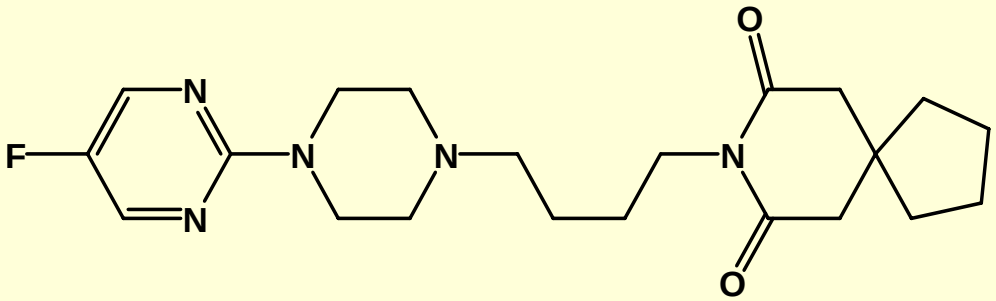
- **Phase I Metabolism**
  - Block the labile sites
  - Remove the labile sites
  - Reduce Log P
  - Add polar functional groups
- **Phase II Metabolism**
  - Add electron withdrawing groups
  - Add steric hindrance
  - Isosteric replacement of OH or COOH

Block Labile Site to Improve Metabolic Stability

	5-HT1A	CYP3A4
	IC_{50} (μM)	$t_{1/2}$(min)
Buspirone	0.025	4.6
	0.063	52.3

Hydroxylation





M. Tandon, et al, Bioorg. Med. Chem. Lett., 14, 1709-1712, 2004

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Lead-Like Compounds

“Lead-like” Properties

- **HTS is commonly used to generate “hits”**
  - Starting points for selection and optimization
  - Companies evaluate HTS hits using Lipinski’s “Drug-like” Rules
    - MW < 500, HBA < 5, HBD < 10, ClogP < 5
- **MW, lipophilicity and H-bonds increase during optimization**
  - Substructures added to increase target affinity
  - Compounds become non-drug-like; exceed Lipinski’s Rules
- **“Lead-like” properties: lower starting values**
  - MW = 100-350, ClogP = 1-3
  - Optimized compounds stay within drug-like range

Lead-like Properties

- **Rule of 3 "RO3"**
  - MW ≤ 300
  - clogP ≤ 3
  - Rotatable bonds ≤ 3
  - HBD ≤ 3
  - HBA ≤ 3
  - (PSA ≤ 60 $Å^2$)
- **Design libraries and select leads based on these guidelines**

Why Select Leads With Good Properties

- The Lead is the structural “template” for optimization
- Optimization phase tends conserve the template
- Template locks in many properties
- Important to select or modify templates for good properties during the hit-to-lead stage
- Start optimization stage with templates having good properties

“Rules” for Rapidly Evaluating Drug-Like Properties

“Rule of 5” or “Lipinski’s Rules”

- **“Poor absorption or permeation are more likely when:**
  - > 5 H-bond donors (expressed as sum of OHs and NHs)
  - MW > 500
  - CLog P > 5 (or MLogP > 4.15)
  - > 10 H-bond acceptors (expressed as sum of Ns and Os)
  - Substrates for biological transporters are exceptions to the rule”
- **Derivation**
  - Compounds surviving Phase I “USAN” (~2200) vs entire WDI (~50,000)
- **Application**
  - Planning synthesis and screening libraries
  - Alert you: potential absorption problems
- **Advantages**
  - Fast; no cost; standard benchmark; well documented; widely used

C. Lipinski, *et al. Adv. Drug Deliv. Rev.* 23:3-25 (1997)

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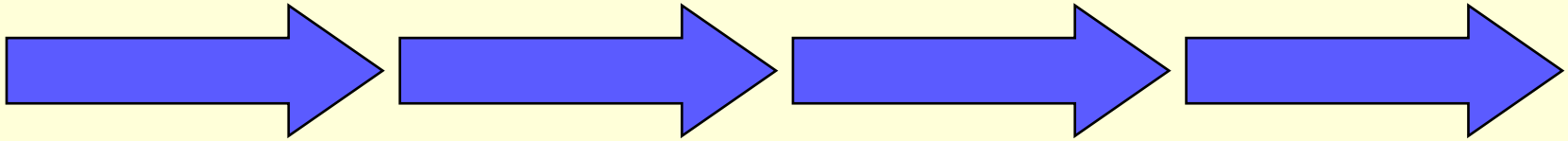
Effects of Properties on Discovery Biology

Each Stage of Biology Has Property Issues

SAR / Efficacy Experiments



HTS	Enzyme Assay	Cellular Assay	Animal to Human
• Identity • Purity • Solubility	• Stability in Bioassay • Solubility	• Permeability • Solubility • Stability in Bioassay	• Met. Stability • Plasma Stab. • GI Stability • Solubility • Permeability • PK • Safety

Consider properties in assay development and data interpretation

Example: Cell-Based Assays Are Affected by Permeability

Compounds	In Vitro Ki (uM)	PAMPA (P_e)	Cell-Based IC 50 (uM)
A	0.007	4.9	10.5
B	0.02	1.0	22.1
C	0.01	0.02	inactive
D	0.05	0.1	inactive
E	3.5	14.3	inactive
F	17	6.6	inactive
G	4.3	0.01	inactive

*Pe values are in units of 10^{-6} cm/sec.

Courtesy of Li Di

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Example: Solubility Affects Activity Assay

Receptor Activity Assay

Initial IC_{50} = 1 μM

Retest IC_{50} = 1 nM (solublized)

1000x increase in activity !

Courtesy of Li Di

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Insoluble Compounds Lead to Erratic SAR

Series	Precipitation after 1st Dilution	Concentration for 30 uM Dose (uM)	SAR
1	0	30	Reliable
2	+++	5.6	Erratic

Courtesy of Li Di

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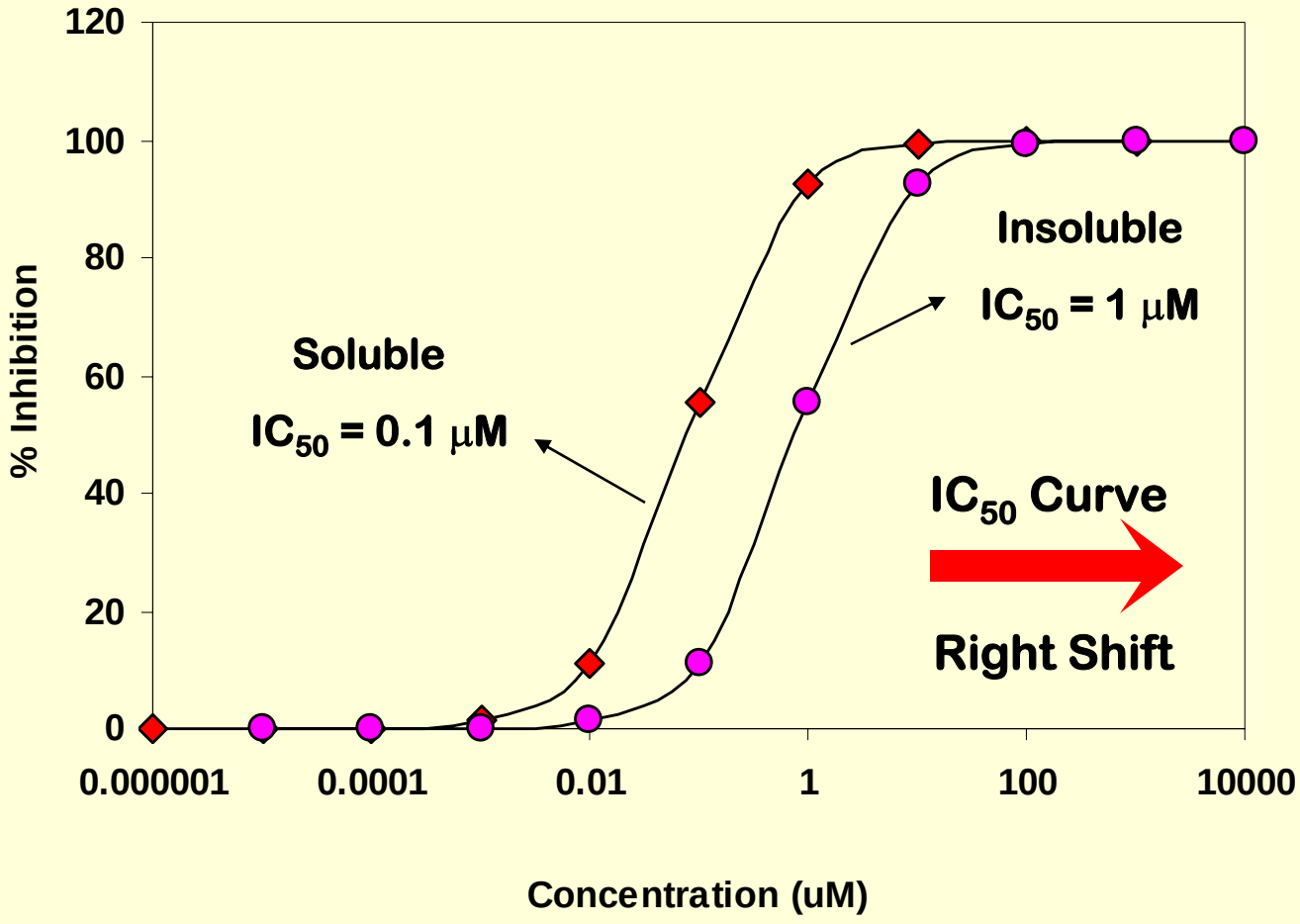
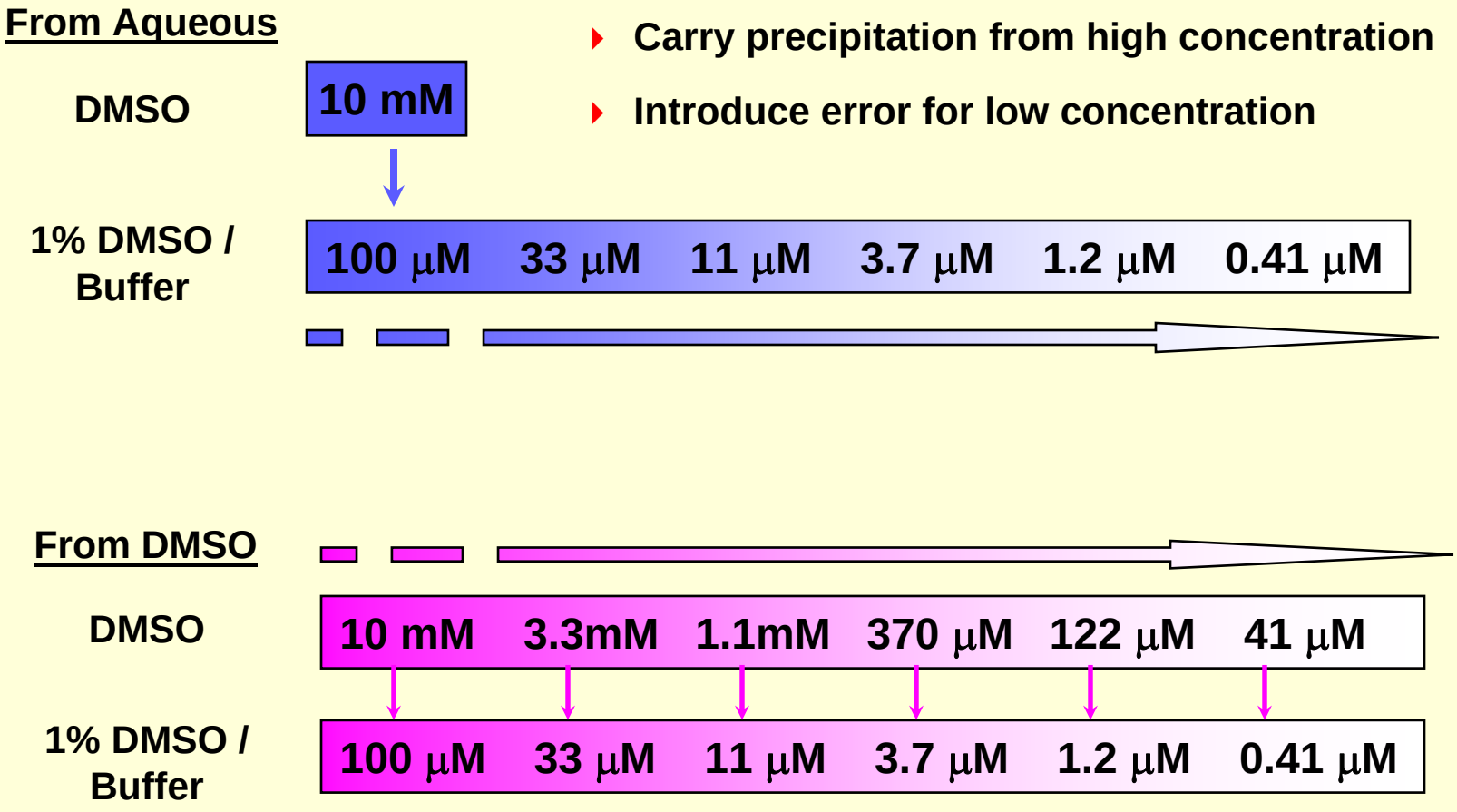
Right Shift of IC_{50} due to Low Solubility

When all the concentrations in assay buffers are lower:



Strategies for Serial Dilution in Biological Assays



- Carry precipitation from high concentration
- Introduce error for low concentration

- High concentration might still precipitate, but will not affect low concentration

Serial dilution from DMSO is preferred

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Conclusions: Integrate Drug-Like Properties into Discovery

- Poor properties can cause failure
- Structure determines properties
- Poor properties causes poor PK
- Assays available for properties
  - Rules, *In silico*, *in vitro*, *in vivo*
- Optimize properties in parallel with activity
- Modify structure to improve properties
- Properties also affect *in vitro* bioassays

Resources for Drug-Like Properties

- ACS Short Course: Drug-Like Properties
- Elsevier Book: January 2008
- AAPS: Drug Design and Discovery Interface Group
- American Chemical Society: Medicinal Chemistry Division

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